1199-Plat

A Transformation for the Mechanical Fingerprints of Complex Biomolecular Interactions

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Biological processes are carried out through conformational transitions, ranging from the structural changes within biomolecules to the formation of macromolecular complexes and the associations between the complexes themselves. These transitions cover a vast range of timescales and are governed by a tangled network of molecular interactions. The resulting hierarchy of interactions, in turn, becomes encoded in the experimentally measurable "mechanical fingerprints" of the biomolecules, their force-extension curves. How can we decode these fingerprints so that they reveal the kinetic barriers and the associated timescales of a biological process? Here, we show [1] that this can be accomplished with a simple, model-free transformation that is general enough to be applicable to molecular interactions involving an arbitrarily large number of barriers. Specifically, the transformation converts the mechanical fingerprints of the system directly into a map of force-dependent rate constants. This map reveals the kinetics of the multitude of rate processes beyond what is typically accessible to direct measurements. With the contributions from individual barriers to the interaction network now "untangled", the map is straightforward to analyze in terms of the barriers and timescales.

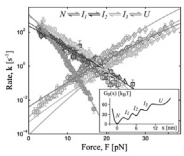

Fig. A rate map obtained by applying the transformation to the mechanical fingerprints of a system with a cascade of barriers along the dissociation pathway.

[1] Y. Zhang and O.K. Dudko, PNAS 2013, doi:10.1073/pnas.1309101110.

1200-Plat

Long-Range Correlated Motion Changes with Protein-Ligand Binding

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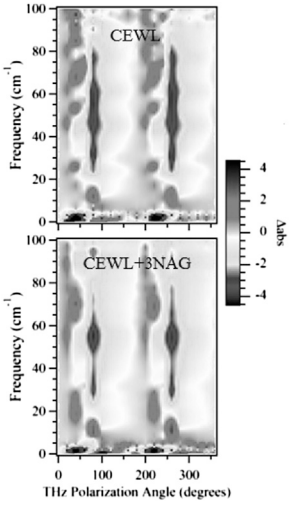
Molecular dynamics calculations have long predicted large scale protein structural vibrations lie in the terahertz (THz) frequency range (5-100 cm^{-1}), and these vibrations are related to protein function. Measuring these vibrational modes has been challenged by the large glass-like contribution from solvent and side chain librational motions. We remove this isotropic background, using anisotropic THz near field microscopy measurements of protein crystals. The technique reveals for the first time narrow band protein excitations in this frequency range. To determine if these features arise from the internal molecular motions, we measured ligand binding dependence using a faster data acquisition technique. The measurements performed on tetragonal chicken-egg white lysozyme (CEWL) single crystals and tetragonal CEWL tri-N-acetylglucosamine inhibitor bound crystals (CEWL+3NAG) show reproducible spectra that change dramatically with inhibitor binding. The large shifts observed indicate the features arise from the protein intramolecular motions and not from crystal phonons, which would have frequency shifts of only $\leq$ 2% with binding. The results validate that the technique can be used to determine ligand binding for inhibitor screening and to understand the role of intramolecular motions in protein function. This work supported by NSF MRI^2 grant DBI295998.



1201-Plat

Proteome-Wide Characterization of Protein Localization Dynamics in Escherichia Coli

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Bacteria exhibit a surprising complexity of subcellular organization despite the absence of membrane-bound organelles and cytoskeletal motor proteins. To capture and analyze localization dynamics throughout the cell cycle at a proteome-wide scale, we combine time-lapse fluorescence microscopy and automated image analysis to capture the cell-cycle localization dynamics of nearly every protein in *E. coli* with non-diffuse localization. For each protein, we capture hundreds of complete cell cycles that facilitates both the quantitative analysis of cell-cycle dynamics and cell-to-cell variation in protein localization. Global analysis of the localization patterns not only recapitulates well-established localization patterns, but also reveals many significant variations in localization both spatially and temporally. We briefly explore one new global insight into protein localization that we believe will be of universal interest: Asymmetric partitioning of proteins at cell division. Although cell division in E. coli was long believed to be essentially symmetric, we have discovered a significant number of transcription factors that partition asymmetrically. This observation in E. coli suggests that processes like asymmetric cell division, which plays a central role in development, have primitive precursors in bacterial cells with even the simplest life cycles.

Platform: Membrane Receptors and Signal Transduction II

1202-Plat

Flim- FRET, a Structural Tool for ErbB Receptor Studies in the Living Cell

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The association state(s) and activities of the ErbB receptor family members in intact living cells differ widely depending upon expression levels and their distribution and interaction partners. There are contradictory views in the literature about the aggregation states and presumed structures of the receptors in the cell membrane. Fixation artifacts may account for apparent quantitative discrepancies. We obtained biophysical FRET/FLIM data on living cells that reveal structural features of ErbB1 (EGFR) and ErbB2 as well as the effects of EGF and various kinase inhibitors on these structures.

We constructed transgenes in which an acyl carrier protein sequence was introduced between the signal peptide and the mature receptor protein sequence. ACP-ErbB1 behaves similarly to wild type ErbB1 with respect to EGF binding, activation and internalization. The ACP-ErbB2 lacks the capacity for binding ligands but can be transactivated as a heterodimer with ErbB1 or ErbB3. Enzymatic labeling of the specific serine in the ACP tag by fluorescent CoA substrates served as donors. The FRET acceptor was the novel membrane probe, NR12S, which is confined exclusively to the outer leaflet of the plasma membrane.

Addition of NR12S to the cells led to a dramatic reduction in the fluorescence lifetime of the donor, indicating a close proximity of the N-terminus of the ErbB1 ectodomain to the plasma membrane, supporting the published autoinhibited structure. EGF addition caused a time-dependent increase in the donor lifetime (reduced FRET), in accordance with the extended dimeric ectodomain structure oberved by Xray-crystallography. The effects of kinase inhibitors on these states and on ensuing endocytosis were also studied. The influence(s) of ErB2 density and antibodies interfering with receptor dimerization were additional topics addressed in this study. TCSPC lifetime images were analyzed with Mathematica software developed for these studies.

1203-Plat

Clustering of H-Ras on the Plasmamembrane of Living Cells

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The composition of the plasma membrane has long been modeled as a fluid mosaic. Studies in the last few years have identified microdomains like lipid rafts and caveolae and membrane-skeleton related fences that constrain membrane proteins within a small region of the cellular plasma membrane. These domains facilitate anchoring of different signaling proteins, like the Ras family of proteins, that has been shown to co-localize with nano domains upon activation by single-molecule tracking studies. It is believed that these nanodomains function as important platforms for a multitude of signalling cascades that are initiated at the plasma membrane. Given that many of the transmembrane signals will need a coordinated domain organization, it is of importance to investigate properties like size, shape, stability and their mutual interaction in a live-cell setting.

Here we transfected 3T3-cells to express the membrane anchors of H-Ras, N-Ras and K-Ras, respectively, when fused to the photoswitchable protein mEos2. Photo-activated localization microscopy (PALM) was used to make super-resolution images of Ras-anchor distributions on the apical membrane of the cells. The spatial distributions were tested against a homogeneous distribution by means of Ripley's analysis. Data showed that for all membrane anchors, the distributions deviated significantly from purely

random. Domains were directly observed. The domain size depended on the type of the membrane anchor (HRas: 130 nm, KRas: 200 nm; NRas: 200 nm). Further we found that domain formation and disassembly was dynamic on a timescale of 5-30s.

1204-Plat

Super-Resolution Localization Microscopy Identifies Distinct Stages of Antigen-Induced IgE Receptor Cross-Linking and Immobilization in Rbl-2H3 Mast Cells

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Cross-linking of immunoglobulin E (IgE) bound to its receptor, FcεRI, by multivalent antigen initiates a transmembrane signaling cascade essential for mast cell activation and important for inflammatory immune responses and allergic disease. In this study, we apply super-resolution fluorescence localization microscopy to record receptor organization and dynamics on live RBL-2H3 mast cells undergoing antigen-mediated signaling, allowing us to measure nanoscale clustering and diffusion of FcεRI simultaneously. Through comparison of cross-linking-induced changes in these properties as a function of time, we are able to resolve two distinct temporal phases of receptor clustering and immobilization. Additionally, we correlate the time-dependence of the distinct phases with a functional signaling response, Ca^{2+} mobilization. In the first phase of receptor clustering and immobilization, receptors slow dramatically with a relatively small average increase in clustering, and individual receptors appear to transiently associate with small clusters. This first phase occurs before Ca^{2+} mobilization and concurrently with initial signaling steps. At later times, receptor-rich clusters become increasingly dense while receptors remain predominately immobilized. These latter behaviors are observed at times following the initiation of the Ca^{2+} response, and we conclude that although cross-linking is necessary for commencement of downstream signaling, receptor assembly into large, densely packed clusters at later times is likely associated with termination of the stimulated response. These findings motivate future study of the physical interactions that give rise to the observed changes in receptor organization and mobility, and how these translate into cellular functions. In ongoing experiments, we are exploring the requirements of signaling for receptor cross-linking through the use of antigens with controlled structure and valency, and we will correlate our observations with functional responses such as Ca^{2+} mobilization and receptor association with downstream signaling partners.

1205-Plat

The Actin Cytoskeleton Controls the Activation of Invariant Natural Killer T Cells by Fine-Tuning CD1d Nanoscale Aggregation on Antigen Presenting Cells

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Invariant Natural killer T (iNKT) cells are a subset of lipid-specific T cells, restricted by the MHC-I class like molecule CD1d. A recent study showed a correlation between the co-localization of lipid-loaded CD1d molecules with lipid rafts on the membrane of antigen presenting cells, and their capacity of eliciting secretion of Th1-cytokines by stimulated iNKT cells [1]. Thus, this study suggested that not only the structure of CD1d bound to an exogenous lipid could influence Cd1d-mediated immunity, but also its partitioning on the membrane. Here, we address the spatiotemporal behaviour of α-Galactosylceramide loaded CD1d complexes on the cell membrane of *human* myeloid cells using multiple colour high-speed single-particle tracking (100 Hz) combined with an iNKT T Cell Receptor-Qdot conjugate as imaging probe. Furthermore, we complement these studies using STED *nanoscopy* to obtain nanoscale images of CD1d spatial organization. Our results indicate a direct role of the actin cytoskeleton in actively segregating CD1d nanoclusters on the cell membrane resulting in an inhibition of the activation of iNKT cells [2]. As a whole, our work proposes a new paradigm of *biophysical* interaction between CD1d presenting cells and NKT cells which deviates significantly from classical MHCI/II complexes and CD8/CD4-T Cells interactions.

[1] J. S. Im et al., *Immunity*, **30**, 888, 2009.
[2] J. A. Torreno-Pina et al., in preparation.

1206-Plat

Multi-Color, Single-Molecule Fluorescence Imaging of GPCR Signalosomes

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G protein-coupled receptors (GPCRs) are the largest superfamily of membrane receptors in the human genome and they are targets for a quarter of all prescription drugs. Activation of a GPCR by an agonist ligand results in G protein-mediated downstream signaling, followed by kinase action and arrestin-mediated desensitization, internalization/sequestration, and recycling. Selective manipulation of these individual steps of the GPCR activation cycle is often desired when creating drugs targeting a given receptor. We are interested in the C-C chemokine receptor CCR5 that is the major HIV coreceptor used in person-to-person transmission. Globally, the HIV/AIDS pandemic has caused nearly 30 million deaths and a similar number of people are currently infected. Certain analogues of the chemokine RANTES/CCL5 are highly potent entry inhibitors against R5-tropic HIV-1 strains, in vitro and in vivo. Three such analogues, 5P12-, 5P14-, and 6P4-RANTES, are particularly interesting because while they differ only slightly in structure they show strikingly different pharmacological profiles (G protein-linked signaling activity, stimulation of receptor internalization). We have recently developed a general, simple, and robust method for stoichiometric, site-specific fluorescence labeling of expressed GPCRs. The method is based on bioorthogonal conjugation of a fluorescent reporter group to a genetically encoded azido group introduced into expressed GPCRs using amber codon suppression.[1] We have adopted a similar strategy for fluorescent labeling of chemokines with azido groups introduced by chemical synthesis. Here we present our progress towards automated, multi-color, single-molecule fluorescence studies of the compositional and conformational dynamics of GPCR signaling complexes ("signalosomes") using fluorescently labeled chemokines and receptors in biochemically defined systems.

[1] H Tian, TP Sakmar, & T Huber (2013) Site-specific labeling of genetically encoded azido groups for multi-color, single-molecule fluorescence imaging of GPCRs. Methods in Cell Biology, 117, in press.

1207-Plat

Dengue Virus Infection Mediated by DC-SIGN

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DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) is a pattern recognition receptor which binds to the mannose or fucose structures present on a variety of pathogens and stimulates diverse immune responses. DC-SIGN forms nanodomains on cell surfaces which are entry portals for viruses including HIV, Ebola, dengue and hepatitis C. In particular, dengue is a mosquito-borne viral infection and has become a rapidly growing global health threat. Many reports have shown that ectopically expressed DC-SIGN enhances productive dengue infection in different human cell types; however, detailed molecular-level studies on interactions between DC-SIGN membrane assemblies and dengue virus (DENV) at the initial binding and internalization stages are lacking. By employing immunostaining, confocal imaging, super-resolution direct stochastical optical reconstruction microscopy (dSTORM) and flow cytometry assays, we show that cell surface DC-SIGN nanodomains are sufficient to capture the small sized DENV (50 nm), leading to efficient virus internalization and productive infection of the host cells. At the initial binding stage, DENV is highly colocalized with cell surface DC-SIGN domains. Internalization of DENV was observed within a few minutes after incubating DENV with cells expressing DC-SIGN, and massive viral particle synthesis was observed at 24h after infection. In contrast, no virus replication was observed on control cells even after 72h of incubating with DENV. The results indicate that DC-SIGN capturing of DENV leads to rapid internalization of the viruses and productive infection thereafter. Furthermore, superresolution dSTORM shows that a single DC-SIGN nanodomain is sufficient to capture single DENV particles. Supported by NIH GM 41402 and NIAID RO1-AI107731.

1208-Plat

How Talin Head Domain and Soluble Ligand Contribute to Integrin αIIbβ3 Activation

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Integrin αIIbβ3 is widely known to regulate the process of thrombosis via activation at its cytoplasmic side by talin and interacting with soluble fibrinogen. Three groups of interactions regulate integrin activation: a set of salt bridges